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An Experimental Study of the Germination of Wheat Seed under Water, as Related to Temperature and Aeration

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PEI-SUNG TANG

A Dissertation

Submitted to the Board of University Studies of the
Johns Hopkins University in conformity with
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AN EXPERIMENTAL STUDY OF THE GERMINATION OF WHEAT SEED UNDER WATER, AS RELATED TO TEMPERATURE AND AERATION*

PEI-SUNG TANG

(WITH FIVE FIGURES)

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* Botanical contribution from the Johns Hopkins University, no. 111.

Introduction

THE PROBLEM

The environmental complex operates conjointly with the internal characteristics of an organism to control physiological activity. The number of kinds of environmental influences that may act on a living thing is of course very large; it may indeed be regarded as infinite, for it includes, among other things, not only all the kinds of molecules and ions that may possibly act to alter the course of physiological activity in any way, but also as many partial ranges of radiation frequency as one chooses to consider. Each kind of influence may act with any number of degrees of intensity and intensity may fluctuate in any number of ways in the period of the life of the organism or throughout any developmental phase. Also, the time intervals considered in studying developmental changes may have any number of different lengths within the life period. Finally, the complex of all the environmental conditions that influence an organism for any time period may have any number of physiological values or capacities because of differences in the manner in which the component influences are combined or are concomitant.

Starting with a given set of internal conditions, as those of a seed placed in culture for germination, the course of development, or whether development shall occur at all, must be considered as determined by the environmental complex and the physiological capacity of the seed operating together. To appreciate the behavior of a germinating seed it is consequently necessary to take into account not only the whole of the influential complex acting from outside the seed but also the capacity of the seed to respond to or to withstand environmental influences. For a given set of internal conditions (kind of seed, health, vigor, etc.) different response patterns are brought into being for different combinations of effective environmental conditions and their various intensities; provided, of course, that the environmental differences considered are sufficiently great and prevail for sufficiently long periods of time to show noticeable corresponding differences in the behavior of the awakening organism.

To approach an experimental analysis of the conditional control of any kind of organism the problem needs to be attacked by the employment of relatively simple systems—the simpler the more promising—and this statement applies to the kind of organisms to be studied as well as to the environmental complexes that are to be employed. It is also desirable, in the beginning, to study physiological processes that lend themselves most readily to measurement and to comparison with respect to process rates and their end results. The general nature of the relations between organisms and their environments has recently been discussed from this view-

point by LIVINGSTON (12). Somewhat the same viewpoint was in part taken by F. F. BLACKMAN (3), in his classic discussion of environmental optima and limiting conditions.

Most students of the environmental relations of organisms have dealt with systems far too complicated for first steps in such analyses. The problems have in many instances involved too many experimental variables and many of these have failed to lend themselves to adequate numerical description. It has also occurred in many cases that the background variables (those not intended to differ in intensity from test to test) were not described sufficiently well to permit satisfactory approach to duplication if an experiment were to be repeated. These conditions of the experimental background might frequently differ from experiment to experiment and might fluctuate during the experiment periods in unknown and consequently unreproducible ways. Also, the several different intensities of the experimental variables themselves (the ones that are intended to differ in intensity from test to test) have been chosen in many instances so as not to represent adequately the whole range of intensities dealt with. To illustrate: only a few different maintained temperatures might be employed in a study of the temperature relations of a physiological process, when many additional temperatures would be needed to supply the points necessary for the construction of the ordinary temperature curve; the non-temperature conditions of the background might be inadequately described and the length of experiment period might not be chosen so as to bring out the time relations in a consistent manner.

The main purpose of the study to be reported in this paper was to attack a relatively simple set of physiological relations by use of a comparatively simple set of variables. The plant material chosen was a stock of wheat seed, whose germination capacity was to be studied in relation to various sets of environmental conditions. Resting seeds are relatively simple organisms in many respects, at least they are much simpler than seedlings or plants in later developmental phases. Wheat seed is known to retain its viability or capacity for germination without great alteration for rather long periods of time and the variability of a lot of pure-line wheat seed only a year or two old has been found to be not nearly so troublesome to the experimenter as is the variability of lots of some other kinds of seed. Although seed germination is a complex process, yet it is much simpler and more easily measured than subsequent phases of plant development and an end point for the germination phase may be chosen so as to be easily identified. Of course it is clear that the farther the developmental process is allowed to go the more complicated must be the environmental relations, and consequently this study dealt with the process of germination only, from the beginning of soaking to the bursting of the seedcoat.

As to environmental conditions, it is of course necessary that all essential ones for the process studied shall be represented. For the germination of wheat seed these are comparatively few. Light is not essential and the very complicated question of light relations is set aside at once by limiting the problem to germination as it occurs in darkness. Water relations are readily dealt with by having the seeds always under dilute nutrient solution of a 3-salt type, which is very simple in comparison with many other media suitable for seed germination. While wheat seed does not germinate well in distilled water, different proportions of the nutrient salts in a dilute SHIVE solution appear to be without marked influence on germination and the growth of very young seedlings, as was pointed out by GERICKE (6). Maintained temperatures are rather easily arranged in experimental studies that do not involve light. Letting the seeds germinate always under weak aqueous solution not only simplifies the question of water relations, as has been said, but it also simplifies the question of the influence of aeration. Natural aeration in a stagnant solution culture is not very vigorous and different degrees of aeration (including stirring of the solution) may readily be arranged by means of a bubbling air stream.

The duration factor, which is always troublesome in experimentation on environmental influence, was simplified by having the longest experiment period only 24 hours, which markedly limited the possible influence of unknown changes in the medium as well as the extent of fundamental changes in the organisms during an experiment. This question of the influence of alteration in the influential conditions is of paramount importance and it would surely require serious attention if the seedlings were allowed to advance beyond the bursting of the seedcoat or if the experiment periods were much longer than the longest one employed in this study. By the use of three experiment periods shorter than 24 hours data were secured which may be useful in case later studies make the 24-hour period appear too long for the other features of the experimentation, especially with reference to unknown progressive changes in the nutrient medium—such as absorption of salts or ions and leaching or excretion of material from the seeds.

Seed germination was chosen as the subject for this study primarily because it constitutes a readily measured physiological process reduced nearly to its lowest terms of complexity. But there are other good reasons for further experimental study of the viability of a lot of seed. Practical methods for seed testing must be based on the principles to be brought out by such experimentation as this and the more we know of germination in general the more rapidly can seed-testing procedure be improved.

Many experimental studies on the relations of more advanced developmental phases of plants involve the use of plant material secured from

seeds—indeed, for higher plants experimental material must be derived from seeds unless cuttings or other means of vegetative propagation are employed. And one of the main desiderata for any kind of experimentation with plants is that the different individuals of the stock of plants used should be as nearly alike as possible. Whenever seedlings or subsequent stages of development are to be employed it is necessary that serious attention be given to the conditions that prevail during seed germination and it appears that one of the most promising ways to procure a lot of reasonably similar plants is to carry out selections based on germination and the very early phases of seedling growth. Thus, the results of a study such as the one here reported may be valuable in a fundamental and very practical way, as furnishing bases for many kinds of experimental study of more advanced phases.

Because seed germination constitutes a conspicuous example of the awakening of plant organisms from a dormant condition, after all physiological processes have been at very low ebb for a long time, this kind of growth is itself of special importance in general physiology.

The present study was carried out at the Laboratory of Plant Physiology of the Johns Hopkins University, from the fall of 1929 to the spring of 1930. In the planning of the experimentation and in the assembling and presentation of the results the writer has been guided and helped by Professor BURTON E. LIVINGSTON, director of that Laboratory, to whose interest this paper is largely due.

EARLIER STUDIES ON SEED GERMINATION

Ever since the earliest days of botanical science seed germination has been studied, sometimes from the viewpoint of physiology but in most cases with regard to agricultural and horticultural practice, and the literature of this general subject is very extensive. Recent contributions on seed germination may be classified in two categories: (1) Studies undertaken chiefly with reference to seed testing, for comparing different lots of seed in regard to their various degrees of viability or germinative energy. To this group belongs the work of HARRINGTON (8) and WILSON (27). (2) Studies on seed dormancy and on special treatments by which the dormant period may be shortened. Here belongs the work of CROCKER (4), ATWOOD (1), HARRINGTON (9) and MORINAGA (19). Most of the more recent studies are more satisfactory than earlier ones, but the interaction of the organism and its environment deserves more thorough attention than has been given to it. Experimentation on seed germination has generally dealt with the behavior of the organisms in relation to rather limited ranges of artificially controlled or measured experimental conditions and little attention has been paid to the other variables, which are of course influential along with the experimental ones.

Several contributions to our knowledge of this subject should be mentioned here, attention being confined to the few papers that deal with germination from viewpoints more or less similar to that of the present study or that furnish information pertinent to the interpretation of the results to be reported in subsequent sections of this paper. In a study of optimal temperatures for seed germination, employing seeds of 18 different plant forms, a series of maintained temperatures and a number of different substrata, HARRINGTON (8) ascertained the temperature and the length of time best suited for testing the viability of the kinds of seed studied. The results show how different kinds of seed differed in promptness of germination and how different temperatures differed in their influence on different kinds of seed. These results are of course referred to the ranges of influential conditions that were included in HARRINGTON's experiments, but it is impossible to gain a clear idea of his prevailing non-temperature conditions because these were not controlled in detail and their description could not be made as specific as would be necessary if these tests were to be repeated.

WILSON (27) studied the germination of wheat seed on moist plaster-of-Paris plates at different maintained temperatures, continuing each test till no further increase in the number of germinated seeds was observed. The number of days required for the earliest and for the latest germination was recorded in each instance. Some of his resulting data are shown below, for five different maintained temperatures.

	10°	15°	20°	25°	30°
Average germination percentage.....	93.5	96	94	90	78
Average no. of days required for earliest germination	5	4	2.5	2	1
Average no. of days required for latest germination	11	10	6.5	4	4

From these data WILSON concluded that the optimal temperature for germination was 15°, because that temperature gave the highest average germination percentage at the end of the experiment. For 15° the final percentage was 96 and the period was 10 days, but nearly as high percentage values were obtained at temperatures of 10° (93.5 per cent. in 11 days), 20° (94 per cent. in 6.5 days) and the final percentage for 25° was of the same order of magnitude (90 per cent. in 4 days). In

studying these results it is surely permissible to consider 93.5, 96 and 94 as alike, and it seems clear that, when we consider the duration factor, 20° must be regarded as much more nearly the general temperature optimum than 15°. At any rate, it is clear from the tabulation just presented that any temperature between 10° and 25° might be expected to give nearly complete germination in a lot of seed like that used by WILSON.

MORINAGA has recently reported a series of studies on germination (19, 20, 21) and those devoted to germination under water are of special interest in connection with the present report. In each of these water experiments the seeds were on the bottom of a 100-ml. Erlenmeyer flask filled with distilled water and kept in a greenhouse at a temperature fluctuating between 15° and 35°, the water being renewed every two weeks. A seed was considered as having germinated as soon as the hypocotyl or plumule showed notable enlargement. Out of 78 plant forms tested, the seeds of 43 germinated under water and 18 of these showed no decided differences between germination in water and germination on wet filter-paper. For the remaining 25 germination was more complete or more rapid on wet paper. MORINAGA reported that his wheat seed was unable to germinate under water, thus agreeing with similar results secured by KRAUS, who found, according to MORINAGA, that his wheat seed did not develop under water farther than to the stage at which the coleoptile protruded. In some of MORINAGA's experiments wheat seeds, as well as seeds of some other kinds that failed to germinate under water, were able to do so when nearly pure oxygen replaced the air above the water. This apparent influence of aeration in connection with seed germination under water is surely important.

In one of MORINAGA's studies (19) 10 samples of 10 seeds each of white clover were tested under water and a similar series of samples was tested on moist filter paper in Petri dishes, at maintained temperatures of 5°, 10°, 15°, 22°, 27°, 32° and 38°. The germination percentages were recorded after 2, 6, and 10 days. The results show a number of remarkable things, some of which were pointed out by MORINAGA. When germinating under water the optimal temperature for this white-clover seed lay between 15° and 27° for all incubation periods studied, but the corresponding filter-paper tests gave a temperature optimum about 22° for a 2-day period and an optimal range of 10°–22° for the longer periods. There was thus a broadening of the optimal temperature range with longer time of incubation when the seeds were on filter paper, a phenomenon apparently related to the shifting of the optimal temperature shown by WILSON's data as well as by those of HAASIS, which will be referred to later on. Had MORINAGA chosen more and shorter time intervals he would probably have obtained a notable downward shifting of the optimal temperature for his filter-paper cultures as the incubation period became longer. The minimal temperature

for germination, for both water and filter-paper cultures was about 5° for the 2-day period and for longer periods it was considerably lower. The maximal temperature for germination was not shown for any period with the seeds under water, being always well above 38° , but it was apparently not much above that temperature when the seeds were on wet filter paper. Furthermore, the rate at which MORINAGA's lot of white-clover seed germinated in water is much more rapid than the corresponding rate for cultures on filter paper.

By studying the germination of seeds under water, MORINAGA certainly advanced a great step beyond earlier workers in this field. His moisture and aeration conditions were thus much more definitely specified than is possible with other methods of treatment. Since his incubation periods were rather long it might have been better to renew the water oftener than at 2-week intervals, but each time the water is renewed in such cultures the seeds are rather thoroughly aerated for a short time. A continuous flow of the liquid medium might be valuable in such experiments, if it could be arranged, and that might care for the maintenance of aeration conditions as well as of other solution characteristics.

MORINAGA suggested that the differences which he observed between germination in distilled water and on wet paper might be related to oxygen supply and the accumulation of toxic products of respiration. Such products might not accumulate so much in seeds surrounded by liquid medium as in seeds on wet paper; for the liquid medium acts to remove excreted material from the immediate surroundings. The so-called poison action of distilled water may perhaps deserve consideration in this connection. It has been studied by many writers, among them LOCKE (16), RINGER (23), OSTERHOUT (22), LIVINGSTON, *et al.* (14), TRUE (26), MERRILL (18) and GERICKE (6). There seems to be no doubt that a weak nutrient solution is, in general, more satisfactory as culture medium than distilled water is. Distilled water is never quite pure and it may influence an organism in contact with it by supplying the absorbing cells with influential ions or molecules in extreme dilution, as Cu or Zn or organic substances that distill with steam. This possibility is difficult to avoid. Distilled water also differs from a nutrient solution in that it furnishes no considerable diffusion tension of the essential inorganic ions and therefore generally permits more loss of substances from the organism than would occur if a good nutrient solution were employed. Finally, ions and molecules leached from the absorbing cells may accumulate in the medium and, with or without modification, these may subsequently exert considerable influences upon the organism. Leaching appears to occur more rapidly when seeds are under distilled water than when they are under a fairly well balanced nutrient solution.

HAASIS (7) studied the germination of seeds of several species of coniferous tree with special reference to the influence of incubation temperature and length of the incubation period. He emphasized the importance of defining or specifying all the influential background conditions of an experiment, although these do not enter directly into consideration as experimental variables. He also emphasized in a new way the importance of the time factor in physiological processes. His results with pitch pine seed are specially interesting in the present connection. Employing an extensive series of maintained temperatures ranging from 16° to 57° and incubation periods ranging in length from 6 or 7 hours to 14 days, he observed that the optimal temperature for the germination of his pitch pine seed shifted from about 47° (for a 1-day period) to about 23° (for a 10-day period) apparently remaining unchanged for more prolonged incubation. With incubation periods of intermediate length there was evidence of two very different optimal temperatures, one at about 31° and the other at about 43° . This important observation would have escaped attention had the incubation periods of about 2 or 3 days been omitted from the plan of HAASIS's study. The same writer also emphasized for the first time the possibility of using a series of different germination treatments for the purpose of defining and sorting out several physiologically different classes of seeds from a given lot. HAASIS's technique did not allow very definite specification with regard to moisture and aeration conditions. All of his seeds were treated with hydroxy-mercuri-chloro-phenol ("semesan") to avoid mold growth. His experimentation was unusually well planned and his data for pitch pine seed are exceptionally complete and generally very consistent. Many interesting points are brought out in his discussion.

Methods and procedure

THE MAINTAINED TEMPERATURES

For maintaining the desired temperatures the series of seven chambers described by LIVINGSTON and FAWCETT (15) was employed. The apparatus stands in one of the greenhouse rooms of the Laboratory of Plant Physiology of the Johns Hopkins University. The chambers are vertical cylinders 28 cm. in diameter and 43 cm. high, each with a water jacket and a stirrer to keep the water in slow rotation. The whole series is well insulated from the outside air by means of packed ox hair and wood and each chamber has a removable lid made of wood and cork. Adjacent water jackets are separated from each other by vertical partitions of galvanized and asphalted sheet iron. Water does not move from one jacket to the next but heat is transferred through the partitions being supplied at one end of the series by means of a thermostatically controlled electric heater and removed at the other end by means of an electrically driven and auto-

matically controlled mechanical refrigeration machine. A thermal gradient is thus maintained between the two ends of the series and each of the seven chambers has its own maintained temperature, which fluctuated, in the present study, within a range of about plus or minus 1° C. Proceeding from the warm to the cool end of the row, each chamber is cooler than the next succeeding one. By suitable adjustment of the heating and refrigeration controls different series of maintained temperatures may be secured. For this study the temperatures employed were 12° , 19° , 24° , 30° , 35° , 40° and 45° C. The continuous, slow rotation of the jacket water around each chamber gives to all sides of the chamber practically the same temperature; the lower part of the air mass enclosed in a chamber was found to be generally a little cooler than the upper part but the difference between bottom and top was not over 2° . The culture flasks stood upright at the bottom of the chambers, all at the same level, accompanied by a thermometer or a thermograph, as space allowed.

GERMINATION FLASKS AND ACCESSORIES

Erlenmeyer flasks, of "Pyrex chemically resistant" glass 15 cm. high and with bases 9 cm. in diameter, were used in these germination tests, each flask containing 100 ml. of nutrient solution. The solution was 2.3 cm. deep and the diameter of its circular free surface was 8.5 cm., where it was in contact with the air. For the tests with flowing air each flask was closed by means of a 2-hole rubber stopper bearing an inlet and an outlet tube (inner diameter, about 5 mm.), the inlet extending downward nearly to the flask bottom while the outlet terminated just below the stopper. Three centimeters above the stopper each tube was bent at a right angle, with the horizontal arm 3 cm. long. In operation, each inlet tube was joined to the air-supply tube from its own wash bottle (in the same chamber with the germination flask) and all outlet tubes were joined to a line of tubing leading to an aspirator. All tubing was of glass, with short couplings of rubber tubing. In most of the experiments air from the greenhouse was continuously bubbled through the nutrient solution in each germination flask. At the end of each test the germination flasks were washed with cleaning fluid, thoroughly rinsed with distilled water and allowed to dry by draining before being used again.

AERATION OF CULTURES

For each germination flask with air flow there were two wash bottles of water, so connected that the air stream entering the flask had bubbled through them in series. The first of these (outside of the chamber) was a 200-ml. bottle provided with rubber stopper and two tubes, for inlet and outlet. The inlet tube opened into the greenhouse and the outlet led to the second wash bottle (inside the chamber). The latter was a 1-quart

“Mason” jar, with rubber stopper bearing inlet tube and outlet tube, the inlet leading from the first wash bottle while the outlet led to the flask. The first of these bottles was half filled with water and served primarily as a telltale, used in ascertaining from time to time the rate of air flow in terms of the rate of bubbling; it also served to increase the humidity of the air stream while the latter was still at greenhouse temperature. The second wash bottle (within the chamber) was about half full of water, at the maintained temperature of its chamber. This bottle served to bring the air stream to the desired temperature before it reached the germination flask. The air entering the flask was found always to have the temperature of the chamber in which the flask stood, even with the most rapid rate of air flow used and with the lowest and highest temperatures employed. In passing through the second wash bottle the flowing air was automatically brought to approximate vapor-pressure equilibrium with the dilute nutrient solution in the flask, so that evaporation or condensation in the flask was at a minimum.

The tube leading from each flask was provided with a special orifice, just outside the temperature chamber, for the controlled application of suction from the suction tank—on the principle that rate of gas flow is determined by pressure gradient and orifice resistance. This device was similar to the one used by HUTCHINS (10) for a like purpose, consisting of a plug of cotton wool and pulverized kaolin suitably packed in a slightly conical tube. Each plug was adjusted, by the proper degree of packing, to give the desired rate of flow with the suction employed. To prevent excessive condensation of water in the plug, which might increase its resistance to air flow, HUTCHINS kept the plug temperature always somewhat higher than that of the entering gas, but the same end was attained in the present experiments by means of a drying tube with calcium chloride, inserted between flask and plug inside the chamber. Five different orifice settings were used, with air-flow rates of about 1, 3, 6, 15 and 30 liters per day, or 40, 125, 250, 625 and 1250 ml. per hour. The corresponding numbers of bubbles passing through the telltales were 4, 12, 24, 60 and 120 per minute. The orifices were nearly alike for all flasks in the same experiment and the rates of bubbling in the flasks were similar to those shown by their respective telltales. The discharge tubes all led out of the temperature chambers to the suction tank, a 5-gallon bottle with rubber stopper, inlet tube and outlet tube, the latter leading to a “Cenco-Harrington” filter pump attached to the greenhouse water supply.

The nutrient solution used was of very low vapor pressure, its osmotic value being about 1 atmosphere for 20°, and neither condensation nor evaporation of water was observed to have occurred in any of the flasks, even at the end of the longest experiment period and with the most rapid

rate of air flow and the highest and lowest temperatures employed. This indicates that the second wash bottle in the air line leading to each flask operated effectively as both temperature and humidity control for the air stream. The surface level of the solution in the flasks was consequently always about the same (about 2.3 cm. above the flask bottom) and the submerged seeds were always at practically the same depth. The volume of solution around and above the seeds altered slightly, of course, through absorption and swelling, but water absorption and swelling by such seeds as wheat are almost equal in respect to volume. It is interesting to note that a hundred seeds had absorbed about 2.5 ml. of water when swelling was complete and that the volume of a swollen seed was about twice as great as its original volume before soaking. The total volume of seeds and water decreased by about 0.4 ml. in the first 24 hrs. of soaking. There were apparently some very small gas-filled spaces in the dry seeds, which became filled with water as soaking and swelling proceeded.

The air stream continuously supplied oxygen to the solution across the bubble surfaces and the free surface of the liquid. Across these same surfaces it continuously removed carbon dioxide and any other volatile substances that may have escaped from the seeds. Also, the mechanical action of the bubbles kept the solution in circulation above the seeds and around them and the rate of stirring was determined by the rate of bubbling. It is of course impossible to study these three effects of the bubbling gas stream separately without elaborate technique, but they need to be borne in mind in connection with all experiments with air flow.

CULTURES WITHOUT AIR FLOW

Besides the cultures that were artificially aerated by means of the air stream, there were also corresponding cultures without artificial aeration. For these the procedure was the same as that just described excepting that the flask stoppers were without tubes. Aeration of the solution was limited to such interchange between flask and chamber as might take place through the two perforations in the stopper and such very slow mixing or stirring of the solution as might be due to natural convection.

THE NUTRIENT SOLUTION AND ITS USE

The culture medium used throughout these experiments was a 3-salt nutrient solution of a type used extensively by SHIVE (24), being solution R 3.3, S 3.3 of type I, as described in the "Plan" of the Committee on the Salt Requirements of Plants, of the U. S. National Research Council (13), with equimolar partial concentrations of the three salts and a total osmotic value of about 1 atmosphere at 20°. It contained per liter 0.0084 gram-mol. of each of the salts, KH_2PO_4 , $\text{Ca}(\text{NO}_3)_2$, and MgSO_4 . No iron

was added. The salts used were of the Baker Chemical Company's "C.P., analyzed" grade. The water used was derived from a Barnstead still.

Single-salt stock solutions were prepared in a way similar to that followed by SHIVE, enough of each being prepared at the start to last throughout the whole study. The concentrations of the three stock solutions were: KH_2PO_4 , 0.59 molar; $\text{Ca}(\text{NO}_3)_2$, 1.17 molar; MgSO_4 , 1.01 molar.

Slight turbidity appeared in the KH_2PO_4 solution as originally made up and this was removed by filtering, the final concentration of the solution being ascertained by evaporating a sample to dryness, igniting the residue and weighing the latter as KPO_3 —as recommended by TREADWELL and HALL (25, p. 612). The concentration of the $\text{Ca}(\text{NO}_3)_2$ solution was ascertained gravimetrically, by precipitating the calcium of a sample as oxalate, igniting the latter and weighing it as CaO —as recommended by the authors just mentioned (25, p. 81). The MgSO_4 solution was based on the weight of salt used, considering it as $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and no analysis of that solution was made. From the three single-salt solutions supplies of the 3-salt nutrient solution were made up in the usual way, as needed.

The nutrient solution must have altered considerably during an experiment period and it was presumably very different at the end from what it was at the start. Changes must have been brought about through absorption of water and salts by the seeds and through exudation of material from the seeds. After 24 hours at the highest temperatures used the culture solutions were noticeably turbid. The kind and amount of alteration that occurred in any flask must have been somehow a function of the combination of the variables involved, which were by nature more or less interdependent, and any attempt to study the nature and course of such solution changes adequately would be difficult.

THE SEEDS USED AND THEIR PRELIMINARY TREATMENT

The wheat seeds used in this study were of the "Nittany" variety, from a supply received from the Pennsylvania Agricultural Experiment Station in the fall of 1928. Some of this same stock had been used in MACK's study (17) of carbon-dioxide production and growth by young seedlings. No evidence has been encountered to suggest the occurrence of any alteration in the physiological characteristics of this lot of seed between the time it was received and the conclusion of the present study in the spring of 1930. It is therefore probably safe to suppose that MACK's results and those of the present study are comparable with respect to the characteristics of the seed used. The supply was stored in a burlap sack in an attic room, with temperature between 20° and about 25° . In September, 1929, weevils appeared and the entire supply was treated at that time with carbon-disulphide vapor, after which no more weevils were observed.

Sample tests made before and after this treatment showed no influence of the treatment upon the subsequent behavior of the seeds; their viability was apparently not altered at all.

The seeds for each experiment were selected with respect to specific gravity and general appearance just before they were introduced into the germination flasks. About 30 minutes before the start of an experiment approximately twice as many seeds as would be required (about 150 ml. of dry seeds) were placed in a glass pan 15 cm. in diameter and 3 cm. high and enough nutrient solution was added to just fill the pan. After vigorous stirring for a few seconds all floating seeds, comprising about 5 per cent. of the total number, were removed and the remaining seeds (those of greater specific gravity than the nutrient solution) were spread out on filter paper, from which they were counted out in 100-seed samples into 100-ml. Florence flasks, as many flasks being used as there were samples. All broken or otherwise apparently unusual seeds were rejected.

The selected seed samples were then transferred to the several germination flasks in the maintained-temperature chambers. These flasks had been prepared about an hour earlier, charged with nutrient solution, distributed among the seven temperature chambers and made ready for starting the air streams. When the seeds were introduced, the nutrient solution in each germination flask had already attained the temperature of the chamber in which it stood and the seeds must have acquired that temperature very quickly after their introduction. It required only about a minute to set the stopper in each of the germination flasks and start the air stream. The seven cultures of an experiment were started one after another in the increasing order of their temperatures and about 2 minutes elapsed in each instance. Thus the culture in the second chamber was started 2 minutes later than the one in the first chamber, the culture in the third chamber was started 4 minutes later than the one in the first, and so on, the culture in the seventh chamber being started about 12 minutes later than that in the first.

THE EXPERIMENT PERIODS

How many of the hundred seeds in any test were found to have germinated at the end of an experiment period must have depended in general on the length of the period as well as on the maintained temperature, the aeration treatment and the background conditions, the last-mentioned complex being initially alike for all experiments. The four different time periods employed in this study were 6, 12, 18 and 24 hours in length. The shorter periods were not simply observation intervals in the longest period but period length was treated as a definite experimental variable, there being a separate set of experiments for each of the four different periods. At the end of each experiment the germination flasks were re-

moved from the temperature chambers in the same order as that followed in starting the cultures. The solution was decanted and the seeds were spread out in a Petri dish, from which those that had germinated were counted to give the germination percentages. These operations required about 7 minutes in each instance; consequently the culture for 19° was observed about 7 minutes later than that for 12° , etc., and the culture for 45° was observed about 42 minutes later than that for 12° . It is evident that the actual experiment periods were not precisely the same for the different cultures of an experiment, the actual time being generally a little longer than the schedule time, with the difference somewhat greater for higher than for lower temperatures. In no instance, however, did the difference amount to more than about 30 minutes, and the nature of this study does not warrant the application of special corrections for these small discrepancies.

It may be noted that earlier students of seed germination have generally employed observation intervals as parts of a longer experiment period and that the method used in this study is comparatively new for this sort of investigation. The essential differences between these two methods of securing information on the influence exerted by the duration factor in studies of this kind deserves some attention. Let it be desired to secure experimental values for the germination percentage of a lot of seed for time periods of 6, 12, 18 and 24 hours, the environmental complex being considered as the same for all four durations, or at least for the beginnings of all tests. According to the usual procedure (as that followed in HAASIS's recent study, for instance) the cultures would all be continued for 24 hours, the number of seedlings produced at the end of 6, 12, 18 and 24 hours being ascertained by observations at the ends of the several 6-hr. intervals. After the first, second and third observations the ungerminated seeds would be returned to the standard environmental complex for the next interval. The exposure for 12 hours would consequently have to be interrupted at the end of the sixth hour, the exposure for 18 hours would have to be interrupted at the end of the sixth hour and at the end of the twelfth, and the 24-hour exposure would have to be interrupted three times, at the ends of the sixth, twelfth and eighteenth hours. Such interruptions should introduce corresponding fluctuations in the environmental complex, which usually can not be satisfactorily maintained through the short time periods requisite for observation, and these environmental fluctuations are apt to be very difficult to measure and to take into account when the experimental results come to be compared and interpreted. Common practice has been to regard the possible influence of these fluctuations as negligible, which is scarcely permissible unless suitable preliminary tests have shown this supposition to be legitimate.

When duration is treated simply as one of the experimental variables, as was done in these experiments, no interruption of exposure needs to be introduced. No culture is disturbed till the end of its own test period and observations are made only when tests have been completed. So the environmental complex is maintained throughout the experiment period without any fluctuation due to observations, though of course it may fluctuate more or less for other reasons, just as it does between times of observation when the method of intervals is employed. In the present study the relations between duration of exposure and a given set of environmental features are shown by the behavior of four different seed samples, a separate sample of 100 selected seeds being used for each of the four different exposure periods. On the other hand, the commonly used method of observation intervals and interrupted exposures does allow the same individual sample of seed to be exposed for all of the different periods considered, an advantage that is not offered by the procedure followed in this study. Various features of experimental technique, as well as the pertinent logical considerations, will naturally need to be taken into account when it is to be decided which of these two methods for dealing with the duration factor is to be employed for a projected series of experiments.

THE CRITERIA OF GERMINATION AND THE GERMINATION PERCENTAGES

For the purposes of this study a seed was considered to have germinated when the white coleoptile became visible through rupture of the seed-coat. This is the earliest stage of germination that can be readily and definitely recognized by ordinary ocular observation. At the end of each experiment each culture of 100 seeds was examined, as has been said, to ascertain how many seeds had attained this stage, including those that had progressed somewhat farther, and that number was taken as the germination percentage. Each test was repeated four times and each of the final germination-percentage values is the average of five values derived from as many like tests.

The average germination percentage for any particular environmental-durational complex indicates, of course, approximately what fraction of the number of seeds actually tested were capable of germinating with the specified complex. For example, this lot of wheat seed was of such nature that 33 per cent. of the selected seeds germinated, on the average, in 12 hours if submerged for that period under about 2 cm. of the standard nutrient solution aerated by an air flow of 1 liter per day and maintained at a temperature of 30°. No other one of the 168 different treatments tested gave this index value and only two treatments gave corresponding index values having magnitudes between 29 and 37, inclusive. Such physiological characteristics of a lot of seed may well be as valuable as other character-

istics that have been employed in describing lots of seed, such as average size, average weight, average starch percentage, etc. If a lot of seed is to be described in terms of viability (that is, capacity to produce seedlings) physiological indices of this sort will surely prove to be not only useful but in many instances quite essential. The results of this study actually furnish 168 different viability indices for the lot of seed used, based on as many different treatments or environmental-durational complexes.

It is of course also true, in a sort of converse way, that the average germination percentages resulting from a consistent and adequately inclusive set of tests are efficiency indices of the several environmental-durational complexes to which they correspond; any result of measurement defines both the thing measured and the measuring device, the former with reference to the device and the latter with reference to the thing measured. One of the aims of this study was to find out how the lot of seed used would behave or perform with reference to a series of treatments but another aim was to find out what each of the several treatments would do to representative samples of this lot of seed. Either aspect implies the other. The average percentage values are consequently to be regarded as approximate measurements of the capacity or capability of this lot of seed to produce seedlings and at the same time they are approximate measurements of the capacities of the several environmental-durational complexes to permit or call forth the production of seedlings from samples of this lot of seed. In short, we enquire, what can this lot of seed do and what can these complexes do?

As to the significance of the average germination percentages, these refer, of course, to the lot of seed used in this study and to no other lot. Other lots of the same variety or of other varieties might be expected to exhibit different kinds of germination performance. The several averages also refer to the corresponding treatments, as has just been mentioned. With another nutrient solution, with other maintained temperatures than were actually tested, or with other aeration procedures, this lot of seed or any other lot of seed might have performed very differently.

How nearly the several 100-seed samples tested may have represented the stock of seed dealt with and how nearly alike the several samples were among themselves, are important questions best answered by a study of the consistency of the whole system of numerical results. The average percentages would of course have been somewhat more nearly representative of the lot of seed from which the samples were taken if the tests had been repeated a greater number of times or if each sample had included a larger number of individual seeds. The numerical values show that there was considerable variability among the five supposedly like tests, which may indicate either effective degrees of difference between the environmental complexes for the five tests or effective differences between the capacities of the five 100-seed samples; or both sorts of differences may have occurred

together, which is not unlikely. An examination of the average percentage values leads to the conclusion that they are in general remarkably consistent and that the forms of their graphs would not have been very different if more than five tests of a kind had been made or if more than a hundred seeds had been employed in each test.

If any reader is inclined to apply statistical methods to the average germination percentages the way is clear, for all the values are given in table I. It does not appear, however, that anything would be gained by such treatment of these data and the following discussions will be based on the general consistency of the averages, which is generally of a relatively high degree for such studies as this.

GENERAL PLAN OF THE EXPERIMENTS

It was planned to cover uniformly a definite range of environmental conditions and the entire series of experiments involved tests of all possible combinations of the 7 temperatures, the 6 sets of aeration conditions and the 4 period lengths. Each experiment included just 7 cultures, one for each temperature employed but all with the same aeration treatment and the same period of incubation. The entire series of experiments involved altogether 168 different tests, each with 100 seeds that had been selected by observation and with respect to specific gravity, as has been noted. Each of the 168 tests was made five times, making a total of 840 tests. The earlier experiments were conducted singly but in the latter part of the study two experiments were conducted simultaneously. There were no indications leading to any suspicion that the lot of seed used altered in any sensible way during the term of the present study, and the general consistency of the results indicates that the experimental technique was equally satisfactory for all experiments. As a matter of record, the dates of the routine experiments are added here.

No air flow.....	Aug. 30-Sept. 10; Oct. 8-14, 1929.
Flow of 30 l. per day.....	Aug. 30-Sept. 10; Oct. 2-13, 1929.
Flow of 3 l. per day.....	Oct. 13-Oct. 29, 1929.
Flow of 15 l. per day.....	Oct. 30-Nov. 11, 1929.
Flow of 6 l. per day.....	Nov. 12-Nov. 21, 1929.
Flow of 1 l. per day.....	Nov. 23-Dec. 2, 1929.

Besides the routine tests thus far described some special experiments were performed to gain information on questions that arose as the regular numerical results accumulated. A few of these will receive some attention after the routine experiments have been discussed.

Results of routine experiments

THE AVERAGE PERCENTAGES AND THE PRIMARY GRAPHS

All the percentage values from the routine experiments are presented in table I. The four main vertical sections of the table correspond to the four

TABLE I

(GERMINATION PERCENTAGES FOR FIVE SEPARATE TESTS OF EACH ONE OF 168 DIFFERENT CONDITIONAL COMPLEXES, TOGETHER WITH THE AVERAGE PERCENTAGE FOR EACH COMPLEX)

(FOR EACH FIVE LIKE TESTS THE MINIMUM AND THE MAXIMUM ARE IN ITALICS AND THE AVERAGE IS IN BOLDFACE TYPE)

	TEMPERATURE	For 6-HOUR PERIOD			For 12-HOUR PERIOD			For 18-HOUR PERIOD			For 24-HOUR PERIOD		
		PERCENTAGES FOR 5 TESTS		AVERAGE	PERCENTAGES FOR 5 TESTS		AVERAGE	PERCENTAGES FOR 5 TESTS		AVERAGE	PERCENTAGES FOR 5 TESTS		AVERAGE
				<i>per cent.</i>			<i>per cent.</i>			<i>per cent.</i>			<i>per cent.</i>
Cultures without air flow	deg. C.												
	12	3	3	4	7	8	7	10	12	12	10	13	13
	19	5	4	6	15	9	10	17	12	15	20	15	19
	24	4	6	8	10	13	15	22	16	18	23	22	23
	30	7	7	10	12	16	17	19	25	24	28	23	*30
	35	14	10	*14	15	18	17	20	17	16	18	18	18
Cultures with air flow of 1 liter per day	40	10	12	12	16	16	12	13	13	13	18	16	16
	45	8	10	9	8	7	12	10	5	12	8	13	9
	12	4	4	4	12	7	8	11	14	12	14	15	15
	19	4	5	5	9	11	12	24	18	25	48	57	52
	24	12	5	10	15	18	16	63	62	56	85	85	*85
	30	11	11	11	35	42	29	45	44	36	48	54	56
Cultures with air flow of 3 liters per day	35	13	12	*13	19	21	11	20	18	22	26	22	22
	40	10	14	12	11	11	9	14	11	16	11	11	16
	45	7	3	8	8	8	8	7	9	7	8	7	8
	12	5	3	4	12	7	9	9	10	15	12	14	19
	19	10	8	9	15	11	11	21	24	26	20	21	56
	24	13	9	11	21	23	18	58	23	45	46	46	*69
* Maximum average.	30	12	19	16	43	39	32	45	40	67	53	55	67
	35	18	20	*17	21	25	25	28	20	29	28	28	27
	40	16	14	*17	11	16	21	18	15	18	27	13	18
	45	13	13	13	15	13	12	16	10	14	13	10	13

* Maximum average.

TABLE I (continued)

TEMPERATURE	FOR 6-HOUR PERIOD			FOR 12-HOUR PERIOD			FOR 18-HOUR PERIOD			FOR 24-HOUR PERIOD		
	PERCENTAGES FOR 5 TESTS		AVERAGE	PERCENTAGES FOR 5 TESTS		AVERAGE	PERCENTAGES FOR 5 TESTS		AVERAGE	PERCENTAGES FOR 5 TESTS		AVERAGE
<i>deg. C.</i>			<i>per cent.</i>			<i>per cent.</i>			<i>per cent.</i>			<i>per cent.</i>
Cultures with air flow of 6 liters per day	5 3 6 3 3	5 10 7 16 8	4	9 7 9 7 8	19 20 23 16 16	8	19 21 13 15 19	27 45 39 28 27	17	27 26 30 15 25	68 74 66 65 77	25
	8 15 15 8 8	21 10 11 12 18	11	45 38 39 39 38	63 49 53 66	20	83 77 78 75 79	67 67 65 65 60	33	90 90 94 91 95	80 73 79 79 65	*92
	18 21 17 24 15	24 15 22 19 10	*19	26 28 29 27 30	21 26 23 18 21	*58	27 27 33 28 24	21 24 25 19 19	65	32 28 32 29 31	20 29 17 19 27	75
	27 8 12 14 11		18	21 26 23 18 21	19 16 13 12 11	22	21 24 25 19 19	16 16 12 15 16	28	13 21 14 14 12	15	30
			14	19 16 13 12 11		14	16 16 12 15 16	14	22	30 36 29 22 29	90 80 62 71 76	29
Cultures with air flow of 15 liters per day	3 3 7 3 4	9 8 10 6 5	4	11 10 7 9 7	11 10 7 9 7	9	10 13 18 17 12	29 38 50 55 50	14	90 99 96 93 99	*95	84
	17 15 19 13 10	22 20 16 20 16	8	44 42 43 43 43	68 54 67 56 53	17	81 70 83 95 85	90 85 85 75 79	44	36 38 31 20 31	33	33
	22 20 16 20 16	20 23 19 21 19	15	68 54 67 56 53	35 22 31 28 32	*60	90 85 85 75 79	43 30 25 27 31	*83	17 20 20 22 32	22	22
	26 20 21 17 17	5 15 19 18 17	*20	35 22 31 28 32	25 24 28 15 20	30	19 22 22 26 22	16 15 24 11 11	31	10 11 14 20 18	15	15
	20 20 21 17 17		15	25 24 28 15 20	9 19 20 14 12	22	16 15 24 11 11	15	22	31 35 35 22 23	29	29
	5 15 19 18 17			9 19 20 14 12		15			16	94 92 86 71 65	*95	82
Cultures with air flow of 30 liters per day	4 3 3 2 3	9 6 9 9 4	3	6 10 19 8 16	22 19 27 22 24	12	16 22 12 17 12	45 66 57 39 46	12	99 100 95 91 89	93	93
	9 6 9 9 4	12 7 8 12 11	7	22 19 27 22 24	57 59 41 30 42	23	97 92 91 81 59	90 88 91 80 79	51	53 35 34 49 55	45	45
	12 7 8 12 11	19 17 17 20 12	10	57 59 41 30 42	70 81 78 64 75	46	90 88 91 80 79	44 22 33 44 35	84	27 22 21 19 15	21	21
	19 17 17 20 12	20 22 15 12 24	17	70 81 78 64 75	29 34 19 32 28	*74	20 21 23 20 18	13 16 17 12 17	*86	11 19 16 13 14	15	15
	17 13 21 19 24	11 18 15 17 16	*19	29 34 19 32 28	23 23 20 20 14	28	20 21 23 20 18	15	20			
	20 22 15 12 24		*19	23 23 20 20 14	14 14 17 18 12	20			15			
	11 18 15 17 16		15	14 14 17 18 12		15			15			

* Maximum average.

* Maximum average.

periods used and the six horizontal sections represent the different aeration treatments. Each group of 35 values thus represents a single experiment performed five times. For each time an experiment was performed there are 7 percentage values, corresponding to the seven different maintained temperatures. The minimum and the maximum in each horizontal series of five values from like tests are in italics and the average of each five corresponding values is shown in boldface type. There are 168 of these averages. The highest average for each experiment is marked by an asterisk. Of course the temperature corresponding to this highest average represents about the optimal temperature for seedling production in the experiment in question. For some experiments there are two highest averages, of the same magnitude, which of course implies that the optimal temperature for that experiment may be considered as lying somewhere between the two temperatures thus indicated, or else slightly below the lower one or slightly above the higher one.

It may be noted that most low percentage values may be considered as relatively less precise than high ones. To be counted as germinated at the end of any test a seed must have burst its seedcoat, though some additional growth may have occurred. At the end of an 18-hr. or 24-hr. period with good conditions for germination many seedlings had elongated considerably, *i.e.*, their seeds had passed the critical moment of bursting the seedcoats. But no account is taken in table I of this over-growth, since whenever over-growth occurred there are data for one or more shorter periods. Thus, for example, the average percentage value of 67 for 30°, 3 l. and 24 hr. obviously includes the seeds that had germinated in the 18-hr., 12-hr. and 6-hr. periods. But the value (**16**) for the 6-hr. period under these conditions represents no considerable over-growth. Such considerations are clearly cared for if 6-hr. increments of the average percentages are computed by subtraction and that mode of presentation will be employed below (table II).

On the other hand, at the end of any period a seed might not yet have burst its seedcoat, being consequently counted as ungerminated, but the processes leading to germination might have progressed very far; such a seed might have been recorded as germinated if the period had been an hour or two longer. As the percentage value is higher there is less probability of nearly-germinated seeds, since the maximum possible value in any case can not be above 100. In any case this lack of precision can never amount to more than a few units of the average percentage value. Furthermore, it is not to be considered at all in instances where the next longer period showed no increase; for example, the value 27, for 35°, 3 l. and 18 hr. can not lack precision in this respect because the corresponding 24-hr. period shows exactly the same average percentage as is shown by this 18-hr. period.

It is at once apparent from table I that, within the ranges dealt with, all three experimental variables (maintained temperature, aeration treatment and duration of incubation) were influential in determining the percentages and their averages. The *lowest germination percentages* correspond to environmental complexes that had either low or high temperatures, low degrees of aeration or short periods, or two of these features or all three of them together. The actual minimum percentage is 1 (for the third test of 12° without air flow and with a 6-hr. incubation period) and the lowest average percentage is 3 (for 12°, 6 hr. and air flow of 30 l.). Averages below 5 represent all the 6-hr. tests with 12° and all other tests have averages of 5 or above. Averages of 10 or below may be taken as representing the least efficient environmental complexes studied. These are confined to the following combinations: All 6-hr. tests with 12° and all 12-hr. tests with that temperature, excepting the one with air flow of 30 l. (which gave an average of only 12); all 6-hr. tests with 19°; all tests with 24° and without air flow or with air flow of 1 l. or 30 l.; and all 6-hr., 12-hr., 18-hr. and 24-hr. tests with 45° and without air flow or with air flow of 1 l.

The *highest germination percentages* correspond to combinations of 24° or 30° with 18-hr. or 24-hr. periods and air flow of 6 l., 15 l., or 30 l. Average values above 90 are confined to the 24-hr. tests with 24° and air flow of 6 l., 15 l., or 30 l., and with 30° and air flow of 30 l.

THE TEMPERATURE GRAPHS

Many details concerning the relations of the various environmental combinations included in this study are most readily brought out by means of graphs. The average percentages shown in boldface type in table I may be plotted in many ways, only a few of which will be considered in detail here. Attention will be confined to the graphs on which the percentages are plotted as ordinates. These are of three sorts: (A) those on which temperature values are abscissas (24 graphs), (B) those on which aeration indices are abscissas (28 graphs) and (C) those on which the time indices are abscissas (42 graphs). Each of these three groups of graphs may be arranged for convenient study in two ways. For the first group the 24 temperature graphs may be arranged (A 1) in 6 sheaves of 4 graphs each, every sheaf representing a single aeration treatment while each individual graph represents a single length of incubation period; or they may be arranged (A 2) in 4 sheaves (for the incubation periods) of 6 graphs each (for the aeration treatments). The 28 aeration graphs may be assembled (B 1) in 7 sheaves (for the temperatures) of 4 graphs each (for the times of incubation), or (B 2) in 4 sheaves (times of incubation) of 7 graphs each (temperatures). In like manner the 42 duration graphs may be assembled (C 1) in 7 sheaves (temperatures) of 6 graphs each (aeration treat-

ments) or (C 2) in 6 sheaves (aeration treatments) of 7 graphs each (temperatures). All of these sets of graphs may be readily constructed from the data given in table I. A few of them are presented here, in figures 1-4.

Figure 1 gives representative samples of arrangement A 1. Three of the six sheaves are shown, for cultures without air flow and with air flow of

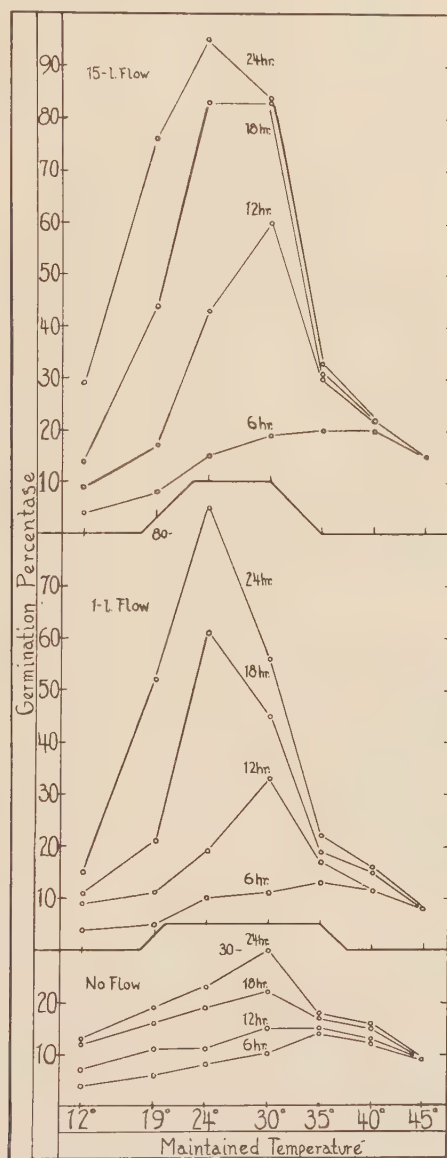


FIG. 1. Representative temperature graphs of germination percentage, grouped according to aeration.

1 l. and 15 l. per day. Each sheaf comprises four individual graphs, one for each of the four incubation periods.

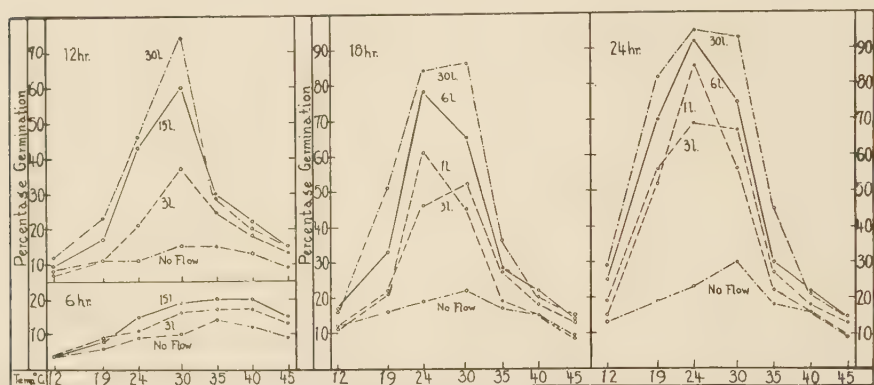


FIG. 2. Representative temperature graphs of germination percentage grouped according to length of incubation period. ("Percentage germination" should be germination percentage.)

Figure 2 presents arrangement A 2, with four sheaves, one for each incubation period. For the sake of clearness one or more graphs are omitted from each sheaf but the general relations are adequately shown.

Some features brought out by these temperature graphs will now be mentioned, reference being made to table I and figures 1 and 2. It is seen that the form of the temperature graph differs markedly according to period length and aeration treatment. All of the 24 graphs agree, as would be expected, in showing relatively low percentage values for 12° and for 45° , with relatively higher values for the intervening temperatures. No minimal nor maximal temperature for germination is shown but the percentages for all tests with 6-hr. period and a temperature of 12° closely approach zero, which suggests that the minimal temperature for germination in 6 hours must have been not far below 12° . For the longer incubation periods the percentages for 12° are progressively higher (for the 24-hr. period, more divergent) and it is suggested that the corresponding minimal temperatures may have been increasingly below 12° with progressively more vigorous aerations as well as with progressively longer periods. The temperature minimum for seedling production by this lot of wheat seed may consequently be said to have been more or less below 12° for all tests, probably farther below that temperature for the tests with longer periods, especially with the more vigorous aeration treatments. For ready comparison, all the average percentages for 12° are shown on page 227.

For the few seeds that germinated at 12° in 6 hr. the aeration differences were apparently without influence, but for those that germinated in longer

	FOR 6-HR. PERIOD, 12°	FOR 12-HR. PERIOD, 12°	FOR 18-HR. PERIOD, 12°	FOR 24-HR. PERIOD, 12°
No flow	4	7	12	13
1-l. flow	4	9	11	15
3-l. flow	4	8	12	19
6-l. flow	4	8	17	25
15-l. flow	4	9	14	29
30-l. flow	3	12	16	29

periods the more vigorous aeration treatments gave appreciably higher percentages. For each aeration treatment the longer the period of incubation the higher is the germination percentage, as would be expected. But the highest germination percentage obtained for 12° was only 29 (for 24 hr. and air flow of 15 l. and 30 l.). It is apparent that no considerably higher percentages than those given would have been obtained for a temperature of 12° combined with any one of these four incubation periods if the air flow had been still more rapid. But it is highly probable that any 24-hr. value for 12° would have been notably surpassed with any one of these aeration treatments if the incubation period had been sufficiently longer than 24-hr.

Turning to the germination percentages shown for the highest temperature employed (45°), all four periods gave the same percentage of germination with all aeration treatments, which indicates that no additional seeds germinated at 45° after the first 6 hr. of incubation, no matter what aeration treatment was employed. It is not likely that a period longer than 24 hr. would have given any additional germination with this temperature for any aeration treatment in the range of treatments studied. This temperature was surely supra-optimal and consequently probably increasingly injurious as time elapsed; those seeds that could germinate quickly under these conditions might do so but those that were delayed more than 6 hr. were unable to germinate at all. This thought is the same as was suggested by HAASIS (7) for a similar state of affairs with his conifer seeds. Although differences in period length did not influence the germination percentage for 45° with any aeration treatment tested, as has just been noted, it is remarkable that the percentage value for this highest temperature combined with any length of period is higher for air flows of 3 l., 6 l., 15 l. or 30 l. than for no flow or for a flow of only 1 l. per day. All the percentage values for 45° are shown on the following page, for comparison among themselves and with those for 12°, given above.

It is indicated that the maximal temperature for germination of this lot of seed was always considerably above 45°, apparently somewhat farther above that temperature with air flow of 3 l. or more but apparently not

	FOR 6-HR. PERIOD, 45°	FOR 12-HR. PERIOD, 45°	FOR 18-HR. PERIOD, 45°	FOR 24-HR. PERIOD, 45°
No flow	9	9	9	9
1-l. flow	8	8	8	8
3-l. flow	13	13	13	13
6-l. flow	14	14	14	15
15-l. flow	15	15	15	15
30-l. flow	15	15	15	15

any higher, for any aeration treatment, with an incubation period of 12, 18 or 24 hr. than with a period of only 6 hr.

With regard to the intermediate temperatures tested (19°, 24°, 30°, 35°, and 40°), all the temperature graphs have the usual form, with a generally well defined graph maximum, the abscissa of which is to be taken as about the temperature optimum in each instance. For any aeration treatment this maximum is always progressively greater with longer periods (fig. 1), as might be expected, and it is generally greater for more vigorous than for less vigorous aeration (fig. 2). The lowest value of this graph maximum is 13 or 14 per cent. (for 6 hr. and 35°, with no flow or a flow of 1 l. per day) and its highest value is 92 or 95 per cent. (for 24 hr. and 24°, with air flow of 15 l. or 30 l. per day). The values for all temperature-graph maxima (maximal ordinates) are shown below, the corresponding abscissa value (about the optimal temperature) being shown in parenthesis in each case.

	GRAPH MAXIMUM FOR 6-HR. PERIOD	GRAPH MAXIMUM FOR 12-HR. PERIOD	GRAPH MAXIMUM FOR 18-HR. PERIOD	GRAPH MAXIMUM FOR 24-HR. PERIOD
No flow	14 (35°)	15 (30°, 35°)	22 (30°)	30 (30°)
1-l. flow	13 (35°)	33 (30°)	*61 (24°)	*85 (24°)
3-l. flow	17 (30°, 35°)	37 (30°)	*52 (30°)	*69 (24°)
6-l. flow	19 (30°)	58 (30°)	78 (24°)	92 (24°)
15-l. flow	20 (35°, 40°)	60 (30°)	83 (24°, 30°)	95 (24°)
30-l. flow	19 (35°, 40°)	74 (30°)	86 (30°)	95 (24°)

As to the general relation of aeration treatment to the maximal percentage value for any length of period, there are four exceptions to the statement that the maximum is greater for more vigorous than for less vigorous aeration: (1) the 6-hr. maximum for a flow of 1 l. (13 per cent.) is not greater than that for no air flow (14 per cent.); (2) the 6-hr. maximum for a 30-l. flow (19 per cent.) is not greater than that for a 15-l. flow (20 per cent.); (3) the 18-hr. maximum for a 3-l. flow (52 per cent.) is

clearly smaller than that for a 1-l. flow (61 per cent.); and (4) the 24-hr. maximum for a 3-l. flow (69 per cent.) is much smaller than that for a 1-l. flow (85 per cent.). The first two of these exceptions may be regarded as not significant but the last two appear to be worthy of careful consideration and their values are marked with asterisks in the above tabulation. They will be reverted to later.

One of the most striking features of the temperature relations here in question is the regression of the optimal temperature with increasing length of the incubation period, for any aeration treatment (fig. 1). It is not quite regular, but there appears to be no doubt of its significance. Other conditions being nearly alike, the optimal temperature for the production of seedlings by this lot of seed is generally lower for longer incubation. If the whole range of period lengths dealt with is considered there are no exceptions to this and the total regression is from about 30° or 35° (or even higher) to about 24°.

A similar regression of the optimal temperature for germination was reported by HAASIS for his conifer seeds. In some cases, as has been said, he found also a double temperature optimum when the length of the incubation period was properly chosen, other influences being the same and favorable to germination, but no indication of a double temperature optimum (bimodal temperature curve) was observed in the present study.

A change in temperature optimum with lapse of time is apparently generally characteristic of growth rates, as has been pointed out by LEHENBAUER (11), TALMA (quoted by BENECKE-JOST, 2, p. 37), FAWCETT (5) and GERICKE (6); but it does not appear to have been emphasized for germination percentage (which is of course fundamentally different from growth rate) excepting by HAASIS. The general question of growth retardation with the lapse of time, when temperature and other influences are maintained, was discussed with characteristic acumen by F. F. BLACKMAN (3).

TEMPERATURE OPTIMA BASED ON AVERAGE HOURLY RATES OF SEEDLING PRODUCTION

To compute the average hourly rate of seedling production corresponding to the highest percentage value for each combination of aeration treatment and duration of incubation we divide each of the maximal percentages by the length of the period that gave it. For example, 6 hr. of a 6-l. air flow gave a maximal germination percentage of 19 (30°) and an average rate of 3.2 seedlings per hour; 12 hr. of a 30-l. flow gave a maximal percentage of 74 (30°) and an average rate of 6.2 seedlings per hour; etc. The average hourly rate of maximal seedling production for all the combinations of aeration treatment and duration are shown on page 230, with the corresponding temperatures in parenthesis.

	HIGHEST AVERAGE HOURLY RATES FOR 6-HR. PERIOD	HIGHEST AVERAGE HOURLY RATES FOR 12-HR. PERIOD	HIGHEST AVERAGE HOURLY RATES FOR 18-HR. PERIOD	HIGHEST AVERAGE HOURLY RATES FOR 24-HR. PERIOD
No flow	2.3 (35°)	1.3 (30°, 35°)	1.2 (30°)	1.3 (30°)
1-l. flow	2.2 (35°)	2.8 (30°)	3.4 (24°)	3.5 (24°)
3-l. flow	2.8 (30°, 35°)	3.1 (30°)	2.9 (30°)	2.9 (24°)
6-l. flow	3.2 (30°)	4.8 (30°)	4.3 (24°)	3.8 (24°)
15-l. flow	3.3 (35°, 40°)	5.0 (30°)	4.6 (24°, 30°)	4.0 (24°)
30-l. flow	3.2 (35°, 40°)	6.2 (30°)	4.8 (30°)	4.0 (24°)

It is seen that this highest average hourly rate was, for each period length, significantly greater with air flow of 6 l., 15 l. or 30 l. than with less vigorous aeration treatments and that for all period lengths excepting the shortest one it was significantly greater with air flow of 1 l. or 3 l. than without any air flow. Furthermore, with air flows of 6 l., 15 l. and 30 l., this index of efficiency has its greatest magnitude (4.8, 5.0, 6.2) for the 12-hr. period and its least magnitude (3.2, 3.3, 3.2) for the 6-hr. period; but this magnitude is about the same (2.8, 3.1, 2.9, 2.9) for all four periods with a flow of 3 l., while it is greatest for the 18-hr. and 24-hr. periods (3.4, 3.5) with a flow of 1 l. Without air flow it is greatest (2.3) for the shortest period.

With regard to average hourly rates the optimal combination of temperature, aeration treatment and time is shown as that for 30°, 30-l. flow and 12 hr., which environmental combination gave the hourly rate of 6.2. The next to the highest rate is 5.0 (for 30°, 15-l. flow, 12 hr.) and a rate of 4.8 is shown alike for the combination 30°, 6-l. flow, 12 hr., and for the combination 30°, 30-l. flow and 12 hr. The highest hourly rate shown for 24° in any combination is very much lower than the ones for 30°; it is 4.6 for the combination of 24°, 15-l. flow, 18 hr., but this same rate is given for the combination 30°, 15-l. flow, 18 hr. and the highest 24°-rate not equalled or surpassed by some 30°-rate is only 4.3, for the combination 24°, 6-l. flow, 18 hr. The optimal temperature for average hourly production of seedlings is clearly 30° and not 24°.

THE AERATION GRAPHS

Figure 3 presents aeration graphs of germination percentage for the 6-hr. and the 24-hr. incubation periods. They are representative of the group of 42 graphs previously referred to as B 2. The graphs for 12°, 19°, 24°, and 30° are continuous lines and those for 35°, 40°, and 45° are broken lines. To save space, two sections of this figure have been cut out, as is indicated, but all points of observation are shown.

The percentage values for the 6-hr. period are all low and about all that can be said of them in general is that more vigorous aeration treatment for

any temperature usually shows somewhat higher percentages, up to an air flow of 6 l. per day, while still more rapid rates of air flow show no significantly greater percentages. No influence of aeration treatment is evident for the 6-hr. period and 12°. For the 6-hr. period and 24° an optimal air flow appears as 15 l. per day and it is suggested that the 30-l. flow may have been supraoptimal for 30° and 35° as well as for 24°. For the 12-hr.

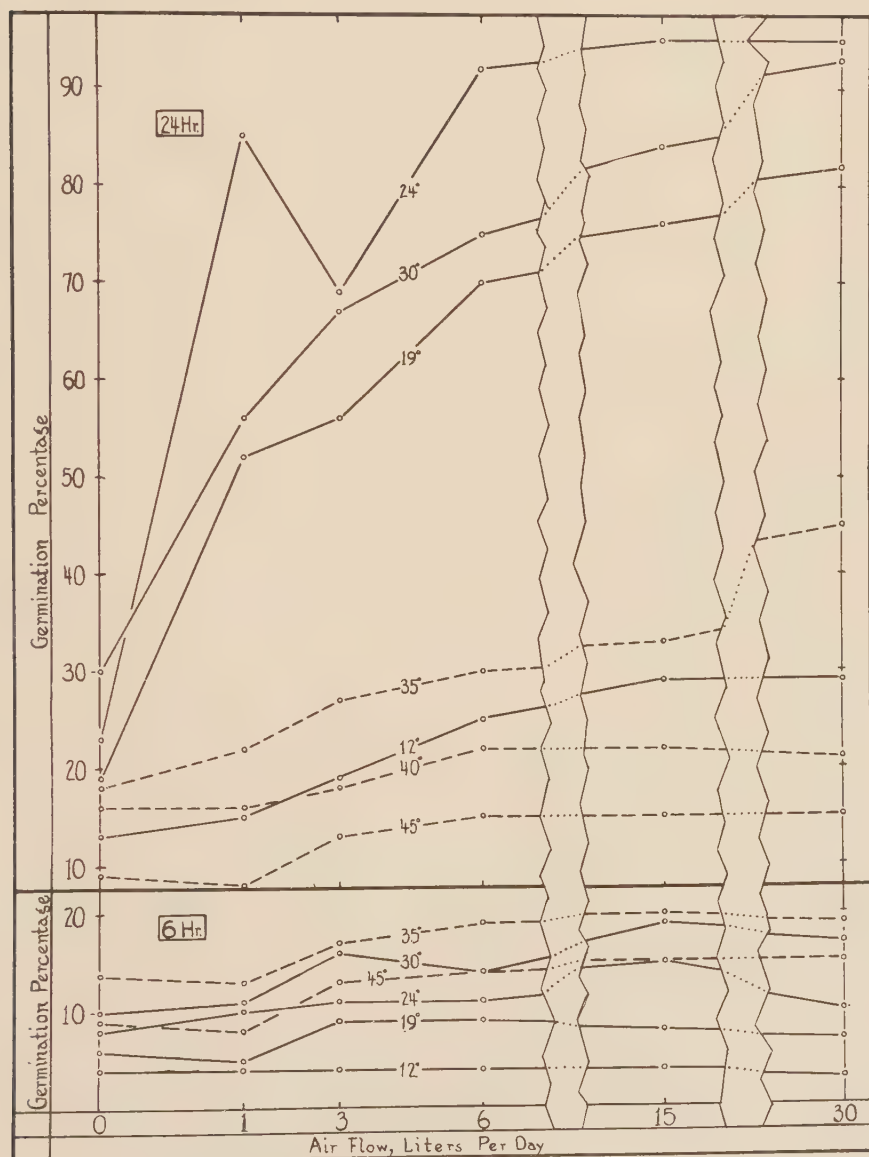


FIG. 3. Representative aeration graphs of germination percentage, for 6 hr. and for 24 hr. of incubation. Two portions are cut out to save space.

period (for which no graphs are shown) no optimal aeration treatment appears for 24° or 30°, and a flow of 30 l. per day gave percentages for the other temperatures just as high as were given for a flow of 6 l. For the 18-hr. period (graphs not shown) the temperatures for which no aeration optimum was attained are 19° and 35°; the 6-l. flow gave as high values as the 30-l. flow for 40° and 45° and the 15-l. flow gave as high values as the 30-l. flow for 24° and 30°.

The sheaf of graphs for the 24-hr. period (figure 3) deserves special attention. They are not very different from those for the 18-hr. period, which are not shown here. The average germination percentage is seen to be generally higher with more vigorous aeration, but for temperatures of 40° and 45° this is not true for air flows more rapid than 6 l. per day, and for temperatures of 12° and 24° the percentage value for the 30-l. air flow is not greater than that for the 15-l. flow. The most consistently upward-sloping graphs are those for 19°, 30° and 35° and for these three temperatures some rate of air flow greater than 30 l. per day might have given a percentage value still higher than the highest one here shown. This suggests that, for these three temperatures, the optimal rate of air flow was not attained, though it appears to have been attained for 12°, 24°, 40° and 45°, within the ranges of conditions here studied. The general air-flow optimum is seen to be the range from 6 l. to 30 l. and any more rapid flow could not have given a percentage much above 93 or 95, since these values closely approach the fixed limit of 100 per cent.

The 24-hr. aeration graph for 24° is remarkable for an apparent secondary minimal point for the 3-l. air flow, which has been mentioned above. This kind of irregularity is definitely shown only for 24 hr., 3 l. (fig. 3) and for 18 hr., 24°, 3 l., but it is suggested by the forms of the graphs for 24 hr., 19°, 3 l., for 18 hr., 19°, 3 l. and perhaps for 12 hr., 30°, 3 l.

This secondary minimal point is of special interest in connection with the double oxygen optima recently reported by МАСК (17) for carbon-dioxide production and shoot elongation in wheat seedlings from the same lot of seed as was used in this study. In the present case it appears that, with a properly chosen maintained temperature (about 24°, which is near the general temperature optimum for this lot of seed) an air flow of 3 l. per day gave a markedly lower germination percentage in 18 hr. or 24 hr. than was given by either less vigorous or more vigorous aeration with the same periods of incubation.

These observations on aeration optima may be important when we shall have learned more of the oxygen requirements of germinating seeds. For many kinds of seed oxygen supply appears to be just as truly important and critical as temperature and water supply are, and aeration of such cultures as those of the present study may be effective—as has been re-

marked—in other ways than through its influence on oxygen supply. To secure very high germination percentages (approaching 100) in 24 hr. or 18 hr., with this lot of seed under dilute nutrient solution, it was only necessary to find the correct combination of temperature, aeration treatment and length of incubation period.

THREE-DIMENSION GRAPH

Another way to bring the average percentages of table I together for somewhat ready comparison, and to show how markedly different environmental complexes gave similar results, is to plot the percentages on three-dimensional diagrams, with contour lines or isopleths connecting points of like value. Since there are four variables to be dealt with and only three may be plotted on a single diagram, several diagrams are requisite to represent all the relations. If we consider the diagrams as representing curved surfaces and always plot the average percentages as altitudes, three sets of diagrams are possible. These may be characterized as follows, the terminology being that of a topographic chart.

SET A.—West-east distances represent the different degrees of aeration treatment and south-north distances represent temperature. There are four diagrams in this set, one for each incubation period. The set for 24 hr. is shown in figure 4 (A).

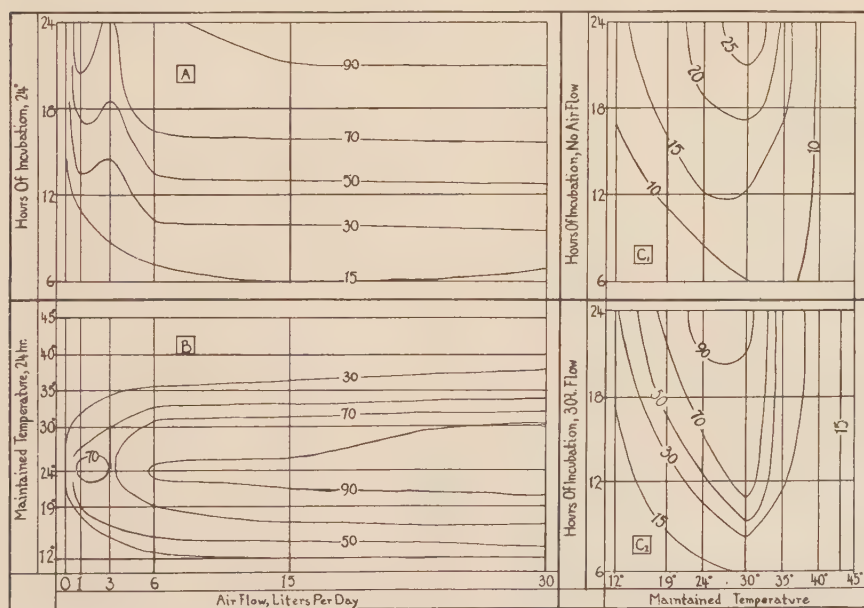


FIG. 4 Representative three-dimension graphs of germination percentage as related to maintained temperature, aeration treatment and duration of incubation.

SET B.—West-east distances represent aeration treatments, as in set A, but south-north distances represent different incubation periods. In this set there are seven diagrams, one for each temperature. The set for 24° is shown in figure 4 (B).

SET. C.—West-east distances represent temperature and south-north distances represent, as in set B, the different incubation periods. There are six diagrams, one for each aeration treatment. The sets for no air flow and for a flow of 30 l. per day are shown in figure 4 (C_1 and C_2).

Each isopleth or contour line of each diagram passes through points of like altitude and all points on any line consequently represent conditional complexes giving about the same average germination percentage. Thus each diagram is seen to be divided into areas by the contours, like a physiographic chart of a terrain. The percentage values are shown on the contours. Each diagram has a "high" and a "low" area and several intermediate ones.

These isopleth diagrams bring out the relations already mentioned and many others in addition, but they need not be discussed in detail. This method of presentation of the numerical values shows that the same percentage value was given by many different treatments of the cultures and that different but equally effective treatments are consistently related to the system of coordinates.

SIX-HOUR AND TWELVE-HOUR INCREMENTS OF THE AVERAGE
PERCENTAGE VALUES FOR THE TWENTY-FOUR HOURS
AFTER THE BEGINNING OF THE EXPERIMENTS

If we consider that the number of germinated seeds in any culture increased with time we may derive 6-hr. and 12-hr. increments by the ordinary method of subtraction. The results of these operations are set forth in table II, which consists of six horizontal sections, one for each aeration treatment. In each section are seven lines, one for each of the seven temperatures, and each line presents, in order from left to right, the increments for the first, second, third and fourth 6-hr. periods, for the first and second 12-hr. periods and the total percentage value for the entire 24-hr. period. The highest value for each period in each section of the table is shown in boldface type and the highest 6-hr. value for each aeration treatment is designated by an asterisk.

The temperature giving the greatest 6-hr. increment value with any air flow is progressively lower for later periods. For example, with air flow of 15 l. per day the optimal temperature for seedling production in the first 6 hours is shown as 35° or 40° , for the second 6-hr. period it is 30° , for the third it is 24° and for the fourth it is 19° . This relation holds for all air flows tested but does not appear to hold for the cultures without air flow.

TABLE II
SIX-HOUR AND TWELVE-HOUR INCREMENTS OF THE AVERAGE PERCENTAGES SHOWN IN TABLE I

AERATION	TEMPERATURE	For 1st 6 HRS.	For 2nd 6 HRS.	For 3rd 6 HRS.	For 4th 6 HRS.	For 1st. 12 HRS.	For 2nd 12 HRS.	For 1st 24 HRS.
Cultures without air flow	<i>deg. C.</i>							
	12	4	3	5	1	7	6	13
	19	6	5	5	3	11	8	19
	24	8	3	8	4	11	12	23
	30	10	5	7	8	15	15	30
	35	*14	1	2	1	15	3	18
	40	12	1	2	1	13	3	16
Cultures with air flow of 1 l. per day	45	9	0	0	0	9	0	9
	12	4	5	2	4	9	6	15
	19	5	6	10	31	11	41	52
	24	10	9	*42	24	19	66	85
	30	11	22	12	11	33	23	56
	35	13	4	2	3	17	5	22
	40	12	0	3	1	12	4	16
Cultures with air flow of 3 l. per day	45	8	0	0	0	8	0	8
	12	4	4	4	7	8	11	19
	19	9	2	11	*34	11	45	56
	24	11	10	25	23	21	48	69
	30	16	21	15	15	37	30	67
	35	17	7	3	0	24	3	27
	40	17	1	0	0	18	0	18
	45	13	0	0	0	13	0	13

* Highest 6-hr. value for the given aeration treatment.

TABLE II (*continued*)

AERATION	TEMPERATURE	FOR 1ST 6 HRS.	FOR 2ND 6 HRS.	FOR 3RD 6 HRS.	FOR 4TH 6 HRS.	FOR 1ST 12 HRS.	FOR 2ND 12 HRS.	FOR 1ST 24 HRS.
Cultures with air flow of 6 l. per day	<i>deg. C.</i>							
	12	4	4	9	8	8	17	25
	19	9	11	13	37	20	50	70
	24	11	29	38	14	40	52	92
	30	14	*44	7	10	58	17	75
	35	19	9	0	2	28	2	30
Cultures with air flow of 15 l. per day	40	18	4	0	0	22	0	22
	45	14	0	0	1	14	1	15
	12	4	5	5	15	9	20	29
	19	8	9	27	32	17	59	76
	24	15	28	40	12	43	52	95
	30	19	*41	23	1	60	24	84
Cultures with air flow of 30 l. per day	35	20	10	1	2	30	3	33
	40	20	2	0	0	22	0	22
	45	15	0	0	0	15	0	15
	12	3	9	4	13	12	17	29
	19	7	16	28	31	23	59	82
	24	10	36	38	11	46	49	95
	30	17	*57	12	7	74	19	93
	35	19	9	8	9	28	17	45
	40	19	1	0	1	20	1	21
	45	15	0	0	0	15	0	15

* Highest 6-hr. value for the given aeration treatment.

Of course this generalization constitutes a step in the analysis of the reasons for the temperature regression already noted.

If we arrange the 6-hr.-increment values that are above 30 per cent. in the descending order of their magnitudes we obtain the following list. Opposite each increment value is shown the particular combination of temperature and air flow that gave it and the number of the 6-hr. period for which it occurs. With the single exception of the value 36, every one of

6-HR. INCREMENT	TEMPERATURE	RATE OF AIR FLOW	NO. OF PERIOD
<i>per cent.</i>	<i>deg. C.</i>	<i>l. per day</i>	
57	30	30	2
44	30	6	2
42	24	1	3
41	30	15	2
40	24	15	3
38	24	30.6	3
37	19	6	4
36	24	34	2
34	19	3	4
32	19	15	4
31	19	1, 30	4

these corresponds to one or another of the three temperature-period combinations, 30°, 2nd period; 24°, 3rd period; and 19°, 4th period. The aeration relations of these best combinations are shown by the upper three graphs of figure 5. In that figure abscissas are aeration treatments and ordinates are 6-hr. germination-percentage increments. Ordinate values are shown by the numerals on the graphs. The graph for 35°, 1st period, is added for comparison.

The three upper graphs illustrate how great may be the difference between cultures without artificial aeration and those with an air flow of 1 l. per day. The cultures without air flow had practically no convection and the oxygen supply to the seeds must have been very slow indeed. Perhaps the marked effect of the slowest rate of bubbling, in giving increment values higher than the corresponding ones without air flow, may have been due to stirring of the solution rather than to the entrance of air into the flasks. A double optimum is again shown on the graph for 24°, 3rd period, and it is suggested for 30°, 2nd period, the increment value for a 1-l. flow being higher than that for a 3-l. flow. For air flows of from 6 l. to 30 l. per day the 6-hr. increment values are about the same on each one of the lower three graphs, but the second 6-hr. period with 30° gave the highest increment (57 per cent.) with the most rapid air flow and might have given still higher values with still more rapid rates of flow.

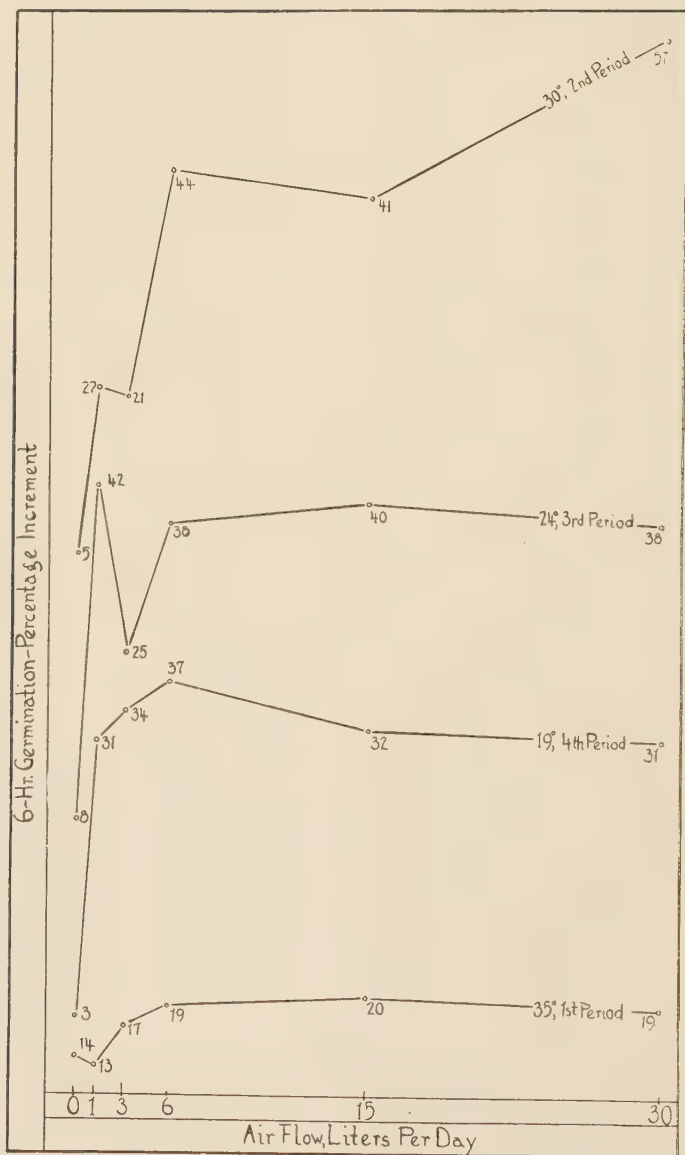


FIG. 5. Representative aeration graphs of the 6-hr. increments of germination percentage. The graphs are arranged to avoid intersections, without reference to the scale of ordinates, but all are plotted on the same scale, ordinate values being shown at the points of record.

If it were desired to select a large number of seedlings as nearly alike as possible, as for a series of solution cultures, about 50 per cent. of this lot of seed might be so selected by taking just those seedlings that had burst the seedcoats in the second 6 hours of incubation under the weak nutrient solution here used and with maintained temperature of 30° and air flow of 30 l. per day. This is an illustration of the manner in which a number of physiologically similar seedlings may be selected by means of differential culture, as has been pointed out by HAASIS. Of course this sort of selection cannot be applied without bringing the seeds into activity and the seeds selected cannot be returned to ordinary storage; they must be allowed to continue their development after it is once started, unless, indeed, they might be held in a kind of dormancy by the employment of suitably low temperature. A study of table II will suggest many ways by which several groups or categories of seedlings might be secured, the individuals of each group being nearly alike by the physiological test of germination in the same short period under a standard set of environmental conditions, while the several groups would differ among themselves in more or less pronounced ways. Seedlings produced in the first 6 hours of incubation with a specified environmental complex might be very different in physiological characteristics from those produced in the fourth 6-hr. period under the same conditions, for example. Whether such differences might be related to genetic characteristics is a question that may be interesting and important.

It will be noted from table II that, for all aeration treatments, germination at 45° was confined to the first 6-hr. period, which has already been mentioned, and that the same is practically true for 40°. With a temperature of 35° most of the germination that occurred is shown for the first 6-hr. period in every case, but the second period gave considerable germination with air flows more rapid than 1 l. per day. With this same temperature still later periods show increments that are nearly or quite negligible, excepting in the case of the most vigorous aeration. These three temperatures (45°, 40°, and 35°) are all above the general temperature optimum for this lot of seed and it appears that they agreed in giving the highest germination percentage for the first 6 hr.

Besides the relations here mentioned, other interesting relations may be noted by a study of table II. The 12-hr. increments of average germination percentage show about the same relations, *mutatis mutandis*, as do the 6-hr. increments and they need not be dwelt on here, for the 6-hr. increments are more useful in studying these relations.

OPTIMAL CONDITIONAL COMPLEXES

From what has been said in the preceding sections it is clear that there are at least three quite different criteria by which we may judge which ones

of the many different combinations of temperature, aeration treatment and incubation period were optimal.

(1) In the first place, most students of germination percentage or seed viability would probably regard as optimal, within the ranges of this study, those sets of conditions that gave the highest average percentages, without regard to time. On that basis, as has been pointed out, we may name three combinations as in the optimal range, namely: 24° , 6-l. flow, 24 hr.; 24° , 15-l. flow, 24 hr.; 24° , 30-l. flow, 24 hr. Because the average percentage values for these three combinations are all nearly 100 (being 92, 95 and 95, respectively) it is possible to say that no other combination of influences could have given percentages significantly greater. Other equally effective combinations might of course be found, especially if the period of incubation were prolonged beyond 24 hr., but these three are surely as truly optimal as any combination that might be tested. Because all three of these optimal combinations are characterized by the temperature value of 24° we have said that the general temperature optimum for nearly complete germination is 24° . And because all three are characterized by the duration value of 24 hr. we may conclude that 24° and 24 hr. are to be taken together in defining a general environmental optimum, together with a rate of air flow of 6-l. per day or more, and the background conditions employed in this study.

(2) In the second place, from another point of view, we may regard as optimal the combination of experimental variables that gave the highest mean hourly rate of seedling production. On this basis, considering the duration factor now, we find that the optimal combination, within the whole range of this study, is 30° , 30-l. flow, 12 hr. This combination gave an average hourly rate of 6.2 seedlings per hour and no other combination tested approached that value more nearly than 5.0. It is apparent that no combination of these background conditions with a maintained temperature higher than 30° might have given a higher hourly rate, nor is there any suggestion that any incubation period shorter or longer than 12 hr. might have given higher rates, unless aeration were still more vigorous than is represented by an air flow of 30 l. per day. It is logically possible, however, that some rate of air flow more rapid than 30 l. per day might have given an average hourly rate of seedling production somewhat greater than 6.2. We may therefore conclude that the optimal complex for this lot of seed, within the parameter of this study, and on the basis of the time rate of seedling production, is either the one noted above or else some combination of 30° with a 12-hr. period and an air flow more rapid than 30 l. per day.

(3) From still another viewpoint, we may regard as optimal, in a special sense, the environmental complex corresponding to that particular portion

of the first 24 hours of incubation which gave the greatest 6-hr. increment in the numerical index of seedling production. On this basis we find an optimal set of conditions defined as follows: 30°, 30-l. flow, 2nd 6-hr. period. This complex gave an increment of 57 per cent. and no other combination tested approached that value at all closely. As by the second criterion (hourly rate), the optimal temperature is here shown as 30°, the optimal rate of air flow is to be considered as 30 l. per day, or else perhaps a still higher rate.

From these and similar considerations it is clear that the optimal temperature for seedling production by this lot of seed, under the background conditions of these studies, may be stated as about 24° or about 30°, according to the criteria used. Either statement may be regarded as correct. This illustrates the principle that no concept of optimal temperature, etc., can be generally clear unless the corresponding or concomitant non-temperature influences and the criteria for judgment are specifically stated.

Special experiments

The results of the routine experiments reported in the preceding section show many combinations of temperature, aeration treatment and length of incubation period that gave average germination percentages smaller than the largest value obtained, which was 95, for 24°, 24 hr. and an air flow of 15 l. or 30 l. per day. It is suggested at once that a large proportion of these low-efficiency combinations were deficient with respect to time only, that they might have given much larger germination percentages if the tests had been continued longer. Circumstances did not permit the longer-period tests that are thus suggested but two like experiments were carried out with an incubation period of 3 days, the seven maintained temperatures regularly employed, and an air flow of 15 l. per day, other details being according to the routine specifications. The average percentage values from these two experiments are given below, along with the corresponding 24-hr.-15-l. values and also the 24-hr.-30-l. values (from table I).

	12°	19°	24°	30°	35°	40°	45°
3-day values, air flow of 15-l. per day.....	95	95	95	87	28	13	14
1-day values, air flow of 15-l. per day.....	29	76	95	84	33	22	15
1-day values, air flow of 30-l. per day.....	29	82	95	93	45	21	15

It is remarkable that the incubation period of 3 days gave the same germination percentage for 12°, and 24° and that this value (95) is the same as the maximum percentage obtained with the combination of 24° and 15 l. or 30 l. and 1 day. It appears that this lot of seed was capable of showing a germination percentage of about 95 with temperatures from somewhat below 12° to about 24° (or perhaps even somewhat higher), with air flow of 15 l. or more, provided the incubation period were sufficiently prolonged. It is also indicated that the combination of 24° and air flow of 15 l. gave no more germination with a longer period than with a period of 1 day; with this combination it appears that all viable seeds had germinated by the end of the 1-day period. Just how long it took to attain the percentage value of 95 with combinations of 12° and 19° with air flow of 15 l. is of course not shown, but that value was surely attained within 3 days of the beginning of soaking. For 30° we may say that the 1-day period and the 3-day period gave about the same percentage value (87, 84) with air flow of 15 l. Whether the value for 1 day and a flow of 30 l. (93) is significantly higher than this may perhaps be questioned and it is at least possible that a period somewhat longer than 3 days might have brought the percentage value for 30° and a flow of 15 l. up to 95. There is obvious experimental discrepancy between the 1-day and the 3-day values for 35° and 40°, but it is at least indicated that all of the seeds that were able to germinate at either of these temperatures had burst their seedcoats by the end of the first day under the specified conditions; for this aeration treatment and either of these temperatures the percentage value is surely no greater for the 3-day period than for the 1-day period. The data for 1 day and 3 days with a temperature of 45° are in excellent agreement; as has been emphasized, all seeds capable of germination at this supra-optimal and surely injurious temperature had apparently burst their seedcoats within the first 6 hr. of incubation, the rest dying eventually.

Cultures held for 6 hr. at 45°, with an air flow of 15 l. per day, and then transferred to 24° with the same aeration for the following 18 hr. gave the same germination percentage as was shown for the first 6 hr. at the higher temperature; about 15 per cent. of the seeds germinated in the first 6-hr. period at 45° and no additional ones germinated in the succeeding 18 hr. at 24°. This supports the conclusion that the seeds that could not germinate in 6 hr. at 45° were probably all dead by the end of that short period.

From a special series of cultures of the sort used in the routine part of this study, with the same seven maintained temperatures and with an air flow of 3 l. per day, the seeds that had burst their seedcoats in the first 6 hr., in the second 6 hr., and in the second 12 hr. were segregated and planted in 21 pots of sand, which were kept in a greenhouse room for four weeks, with daily watering. Most of these lots of barely germinated seeds produced

nearly as many apparently healthy plants as there were seeds in the respective lots, indicating that the seeds had not been injured by the culture treatment employed for germination.

About half of the lot that had burst the seedcoats in 6 hr. at 45° failed to produce plants. This seems to indicate that about half of the germinated seeds from the 6-hr.-45° culture had been seriously injured in that short period, so that they either were dead at the end of the period or died without further development after they had been planted in the pots. The plants that were produced from this lot appeared to be somewhat retarded in their growth and may not have been quite as healthy as those from germination cultures at lower temperatures. Only a very few germinated seeds were available from the cultures at 45° for the second 6 hr. and for the second 12 hr. of incubation, and no plants were obtained from either of these lots. As far as this additional evidence goes, it supports the tentative conclusion reached from the results of the routine experiments, that a maintained temperature of 45°, with the non-temperature conditions of this study, acted injuriously on most of the seeds even in the first 6 hr.

Summary

In this paper are reported results of an experimental study of the germination performance of a lot of pure-line wheat seed (Nittany variety) under different sets of environmental conditions. The study was carried out at the Laboratory of Plant Physiology of the Johns Hopkins University, under guidance of Professor Burton E. Livingston. All apparently imperfect seeds and all seeds that floated on the nutrient solution used were discarded before each experiment was begun. In each germination test 100 seeds lay on the bottom of a germination flask, under 100 ml. of nutrient solution the surface of which was about 2 cm. above the seeds. Seven temperatures (12°, 19°, 24°, 30°, 35°, 40° and 45°) were employed by means of unlighted chambers, for incubation periods of 6 hr., 12 hr., 18 hr. and 24 hr. Six different aeration treatments were employed for each combination of temperature and length of incubation period. By one treatment the cultures were without air flow or agitation, the flask being open to the air of the chamber through an ordinary 2-hole rubber stopper. By the remaining treatments air from outside the temperature chamber was continually bubbled through the solution, renewing the supply of oxygen, removing volatile excretions and insuring the stirring of the solution about the seeds. Five rates of air flow were employed: 1 l., 3 l., 6 l., 15 l. and 30 l. per day. There were consequently 42 different environmental complexes, each being tested with four periods of incubation. The medium was a 3-salt solution containing per liter 0.0084 gram-mol. of each of the salts, KH_2PO_4 , MgSO_4 , $\text{Ca}(\text{NO}_3)_2$. It had a total osmotic value of about 1

atmosphere at 20°. The culture solution was not renewed throughout the incubation period. At the end of each test the number of seeds that had germinated was ascertained and recorded as a percentage of the total number in the flask. Germination was considered as having occurred in all instances where the seedcoat had been ruptured and the white coleoptile was visible through the opening. Each test was performed five times and the five percentages were combined as the average germination percentage for that test.

The results are presented in tabular form and in representative graphs of several sorts. Almost all (95, 92 per cent.) of the seeds of a sample germinated in 24 hr. with the best environmental complexes, and high percentages (86, 83 per cent.) were obtained in 18 hr. under the best conditions. About a fifth of them germinated in 6 hr. under the best conditions.

In general, higher temperatures gave higher germination percentages, up to a temperature optimum, beyond which still higher temperatures gave lower percentages. The optimal temperature was lower as the incubation period was longer, being about 30° or 35° for the shortest period and about 24° for the longest. Aeration treatment exerted little if any influence on the optimal temperature for any period.

For 12°, 19°, 24° and 30° the germination percentages secured with any aeration treatment were progressively higher with longer incubation periods and the highest values were obtained with these temperatures and 24 hr. of incubation. On the other hand, this relation was not so clear for still higher temperatures and did not appear at all for 45°. The seeds that germinated at this highest temperature burst their seedcoats within 6 hr. and longer periods gave no higher values. Aeration treatment was without marked influence on this relation.

In general, progressively more vigorous aeration treatments gave progressively higher germination percentages, up to a rate of air flow of 3 l. or 6 l. per day, but no farther, and this relation was increasingly evident for progressively longer periods. For the longest period (24 hr.) temperatures of 19°, 30° and 35° gave progressively higher values up to the most rapid rate of air flow (30 l. per day) and it appears that still more vigorous aeration might have given higher values than were given by the 30-l. flow. With a temperature of 24° a double aeration optimum is clearly shown for the 18-hr. and 24-hr. periods and such a double optimum is suggested for 19° and 24 hr. and for 19° and 18 hr. The bimodal aeration graph representing this double optimum has a marked secondary minimum corresponding to an air flow of 3 l. per day. That is, for the instances just mentioned rates of flow of 1 l. and of 6 l. per day gave notably higher percentages than were given by the intermediate rate of 3 l. per day.

Some representative 3-dimension graphs are shown, with contours or isopleths passing through points that represent combinations of conditions giving like percentage values.

Average hourly rates of seedling production are presented and discussed, also the 6-hr. increments of average germination percentage.

As to optimal conditional-durational complexes, three complexes gave percentage values above 90, all for 24° and 24 hr., with air flow of 6 l., 15 l. and 30 l., respectively. These combinations of the experimental variables might be taken to represent general optimal conditions. If, however, we consider as optimal the combinations that gave highest average hourly rates of seedling production we must regard as optimal the combination of 30°, 12 hr. and an air flow of 30 l. per day. This gave an average hourly rate of 6.2 seedlings per day and no other combination tested gave a corresponding hourly value approaching this more nearly than 5.0. Furthermore, if we regard as optimal the complex corresponding to that particular 6-hr. interval (of the first 24 hr. of incubation) which gave the greatest 6-hr. increment in the numerical index of seedling production, we should select the second 6-hr. period with 30° and an air flow of 30 l. per day. This complex gave an hourly increment of 57 per cent. which is not at all closely approached by any other corresponding value.

Some special experiments are briefly reported. From an experiment series with an incubation period of 3 days it appears that this lot of wheat seed was capable of showing a germination percentage of about 95 with temperatures from somewhat below 12° to about 24° (or perhaps higher), with daily air flow of 15 l. or more, provided the incubation period were sufficiently prolonged. This may be true for 30° also. For 35° and for 40°, with air flow of 15 l. the 3-day cultures failed to give greater percentage values than were given by single-day cultures. For 45° no more seeds germinated in 3 days than in 6 hr.

Cultures held for 6 hr. at 45° with air flow of 15 l. per day and then subjected to 24° with the same aeration for the succeeding 18 hr., gave a germination percentage of about 15, the same as was given by the first 6 hr. of this test. This supports the conclusion that the seeds that could not germinate in 6 hr. at 45° were probably all dead by the end of that short period.

Seeds that had germinated in the first 6 hr., in the second 6 hr. and in the second 12 hr. of incubation, with air flow of 3 l. per day, and had then been planted in pots of sand and treated for four weeks like ordinary potted plants in the greenhouse, generally showed healthy and usual growth, which indicates that the germination treatment had not been injurious. But about half of the seeds that had germinated in the first 6 hr. at 45° (with 3-l. air flow) failed to continue their development in the pot cultures;

these had been killed in the 6 hr. germination period or else they had been so injured that they died shortly after being planted in the pots.

The results of this study clearly show how the germination performance of this lot of wheat seed was influenced by the experimental variables (maintained temperature, aeration treatment and duration of incubation) and how the influence exerted by any one of these variables was itself influenced by the others. They emphasize again the general principle that environmental influence on organisms needs to be studied and described in terms of environmental complexes rather than in terms of only one or a few of the influential conditions. The influence of any kind of environmental condition or "factor" can be usefully described only with adequate reference to all the other kinds of influential conditions acting at the same time.

It is pointed out that germination with a series of different but simultaneous environmental complexes and different incubation periods might be employed to secure several batches of barely germinated seeds from the same seed stock, the individuals of each batch being rather nearly alike physiologically while the several batches might be nearly alike or notably different, as might be desired. When like batches of seedlings from a given seed stock are needed for experimentation on environmental influences as they affect subsequent developmental phases, such physiological selection as is here suggested may be useful, in addition to the commonly employed selections based on general appearance, size, color, weight, specific gravity, and other morphological characteristics of resting seeds.

The relations here brought out between germination percentage, on the one hand, and the prevailing environmental complexes of the germinators and the duration of incubation, on the other hand, may be useful also in the further working out of standard procedures for ordinary seed testing.

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